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Microscopic Anatomy of the Tentacles of *Thysanocardia nigra* Ikeda, 1904 (Sipuncula) from the Sea of Japan

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Abstract Fine morphology of the tentacular apparatus of the sipunculan *Thysanocardia nigra* Ikeda, 1904 was conducted as a part of a larger study of microscopic anatomy in the species. The tentacular apparatus is composed of two rings of tentacles, the dorsal and oral crowns. The dorsal, or nuchal, crown consists of an arc of nuchal tentacles dorsally enclosing the heart-shaped nuchal organ. The oral disk carries numerous peripheral tentacles arranged in oral crown surrounding the mouth. Peripheral tentacles are arranged in paired rows, additional tentacles being developed posteriorly so as to form a radial series of U-shaped festoons. The parallel festoons extend down the oral disk and run alongside the spherical head. New pairs of festoons appear at the base of the nuchal crown. The tentacles are heart-shaped in cross section, the oral surface being widest. The nuchal tentacles face the oral disk by the oral surface while the peripheral, or oral, tentacles are twisted at the base at nearly right angles, so to face a ciliary groove of the corresponding festoon. The central groove of festoon is composed of a median ridge bordered by longitudinal lateral ridges with long cilia. The oral surface of tentacles is constructed of a multiciliated, pseudostratified, columnal epithelium with some intraepidermal mucous cells. There are three longitudinal tentacular canals lined by peritoneum. Hemerythrocytes flow through the lumen of the canals. Tentacles and grooves of festoons form a filtering system upon which food particles can be trapped. The filtering apparatus of *Thysanocardia* shows remarkable similarities with a filtering system of sabellid polychaetes.

Key words: sipunculan, tentacle, crown of tentacles, filtration, cilium, coelom, nuchal organ

Introduction

Sipunculans, the peanut worms, constitute a phylum of non-segmented vermiform marine coelomates (Rice, 1993). The body is subdivided into two main portions: peanut-like trunk and long narrower introvert. The eversible introvert terminates with a spherical head bearing tentacles. The number of tentacles increases with a size and age of individual. Sipunculan tentacular crown, or tentacular apparatus, exhibits two basic patterns (Cutler, 1994). In the class Sipunculidea, the tentacles are arranged peripherally around the oral disk, encircling the central mouth. The circle is inflected dorsally to form an arc of nuchal crown that encloses the dorsal nuchal organ. In the class Phascolosomatidea, the tentacles are limited to a dorsal arc enclosing the dorsal nuchal organ and there are no peripheral tentacles around the oral disk.

The genus *Thysanocardia* is characterized by the most complicated tentacular apparatus and has a largest number of tentacles within the Sipuncula (Gibbs *et al.*, 1983). The border of the oral disk develops numerous festoons bearing several hundreds of tentacles to enlarge the oral surface of the head (Cutler *et al.*, 1984).

The tentacular crown and tentacular coelom of sipunculans function in gas exchange, feeding process and waste transport (Pilger, 1982; Rice, 1993; Ruppert and Rice, 1995; Adrianov *et al.*, 2001, 2002).

Most sipunculans are deposit feeders and tentacles are used in non-selective deposit feeding to bring sediment and associated food to the mouth (Murina, 1977; Rice, 1993; Cutler, 1994). Some

species are filter feeders with elaborate tentacular crowns and may employ the tentacles in a mucociliary suspension feeding (Pilger, 1982; Cutler, 1994).

Hemerythrocytes, cells bearing the respiratory pigment hemerythrin, flow through a system of tentacular canals (Florkin, 1933; Pilger, 1982; Rice, 1993).

The anatomy of sipunculan tentacular apparatus has been studied in a few species (*Golfingia vulgaris* Cuenot, 1900; *G. elongata* Stehle, 1953; *Sipunculus nudus* Metalnikoff, 1900; *Themiste lageniformes* Awati and Pradhan, 1936). The fine morphology of tentacular apparatus of several species has been well illustrated with SEM methods by Rice (1993) (*Themiste lageniformes*, *Nephasoma pellucida*, *Phascolion cryptus*, *Phascolosoma perlucens*, *Aspidosiphon brocki*).

A structure of tentacular crown in the genus *Thysanocardia* has been briefly described only in two papers, both without electron microscopic methods (see Gibbs *et al.*, 1983; Cutler *et al.*, 1984). The purpose of this paper is to describe in details a fine morphology of the tentacular apparatus in *Th. nigra* using a method of vital observation, scanning electron microscopy and histological methods. This paper is an attempt to provide a structural basis from which a functional analysis of the feeding behaviour in *Thysanocardia* may emerge.

Material and Methods

Specimens of *Thysanocardia nigra* Ikeda, 1904 were collected from the Vostok Bay in the Peter the Great Bay, the Sea of Japan. The worms live between roots of sea grass *Zostera marina* which are present in clusters on sublittoral sand bars at depth of 4-6 m.

Specimens of *Th. nigra* were kept alive in beakers with well aerated sea water for several days before being dissected and processed for electron microscopy. Relaxation of specimens was accomplished by slowly adding 7.5% MgCl₂ to the sea water containing animals. When relaxed to the point that they would not retract the head when agitated, the animals were quickly decapitated. The freed heads were immediately fixed for two hours at 4°C in 0.25 M 2.5% glutaraldehyde buffered in sodium cacodylate, with NaCl and buffered to pH 7.4. The heads were postfixed at 4°C for two hours with 2% osmium tetroxide buffered in 0.2 M sodium cacodylate. After excess fixative was rinsed off with distilled water the tissue was dehydrated in a graduated ethanol and acetone solution series. For SEM, the heads were dried according to the critical-point method, coated with gold and examined in a Hitachi scanning electron microscope. To demonstrate general details a few heads were fixed in Bouins and 10% formaldehyde, dehydrated, embedded in paraplast, sectioned at 5-7 micron thickness, colored with haematoxylin, and mounted on slides. Several complete series of sagittal and transverse sections of *Thysanocardia* heads were mounted and studied with light microscopy.

Results

Thysanocardia nigra has a cylindrical trunk with a long, tapering introvert, frequently one-and-half or even twice as long as the trunk. The gray or brown trunk is with dark brown or black anal and terminal areas. In adult specimens the trunk is commonly 20-45 mm long. The introvert is terminated with a spherical or cylindrical head bearing numerous tentacles. There are no hooks. The tentacles are pigmented in most specimens. The pigment is mauve or yellow in life, fading in preservative to dark brown, and usually appears as a circular patch or band, on each tentacle (Fig. 1).

The tentacular apparatus is composed of two main portions, the dorsal, or nuchal, crown and oral crown (Fig. 1-2). The circular fold of the head, surrounding the oral disk, is inflected dorsally to form a dorsal nuchal arc that encloses the dorsal nuchal organ terminating the head. The arc bears tentacles and forms nuchal tentacular crown. In adults this crown consists of from 12 (6 pairs) to 20 (10 pairs) tentacles (Fig. 1-2). A wide ventral basement of the nuchal crown is situated dorsally and just from above the mouth, pair of midventral nuchal tentacles being just in front to the mouth opening (Fig. 1-2). New dorsal tentacles of grown animal appear in a "growth zone" situated where the circular fold of the head starts to inflect dorsally to form a nuchal arc (Fig. 2 B).

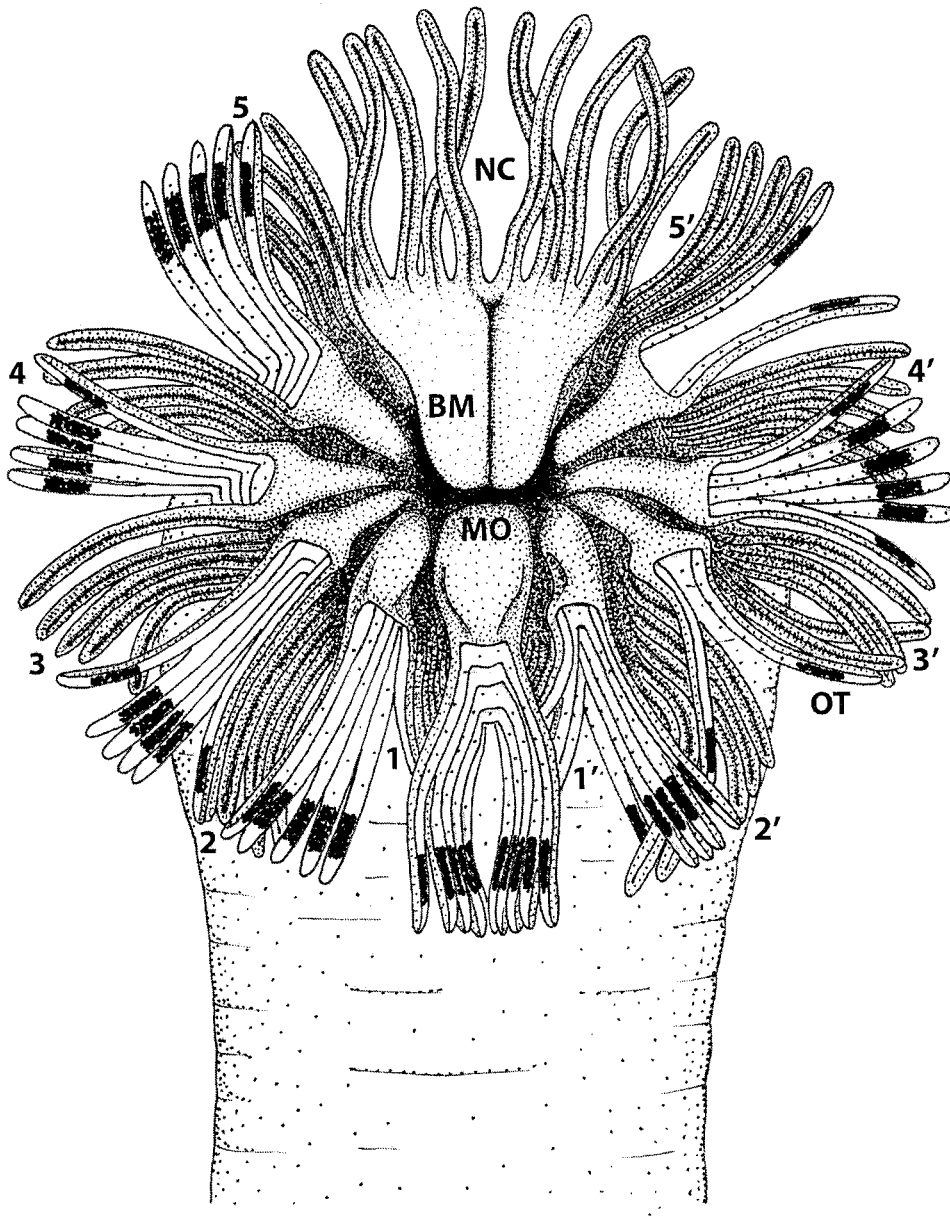


Fig. 1. Head of *Thysanocardia nigra* with 10 oral festoons of tentacles, frontal view. Numbers 1-5 indicate right festoons, 1'-5' indicate left festoons. The dorsal half of 5' festoon is omitted for clarity.

Abbreviations: BM - ventral basement of nuchal (dorsal) crown, MO - mouth opening, NC - nuchal (dorsal) crown of tentacles, OT - oral tentacles.

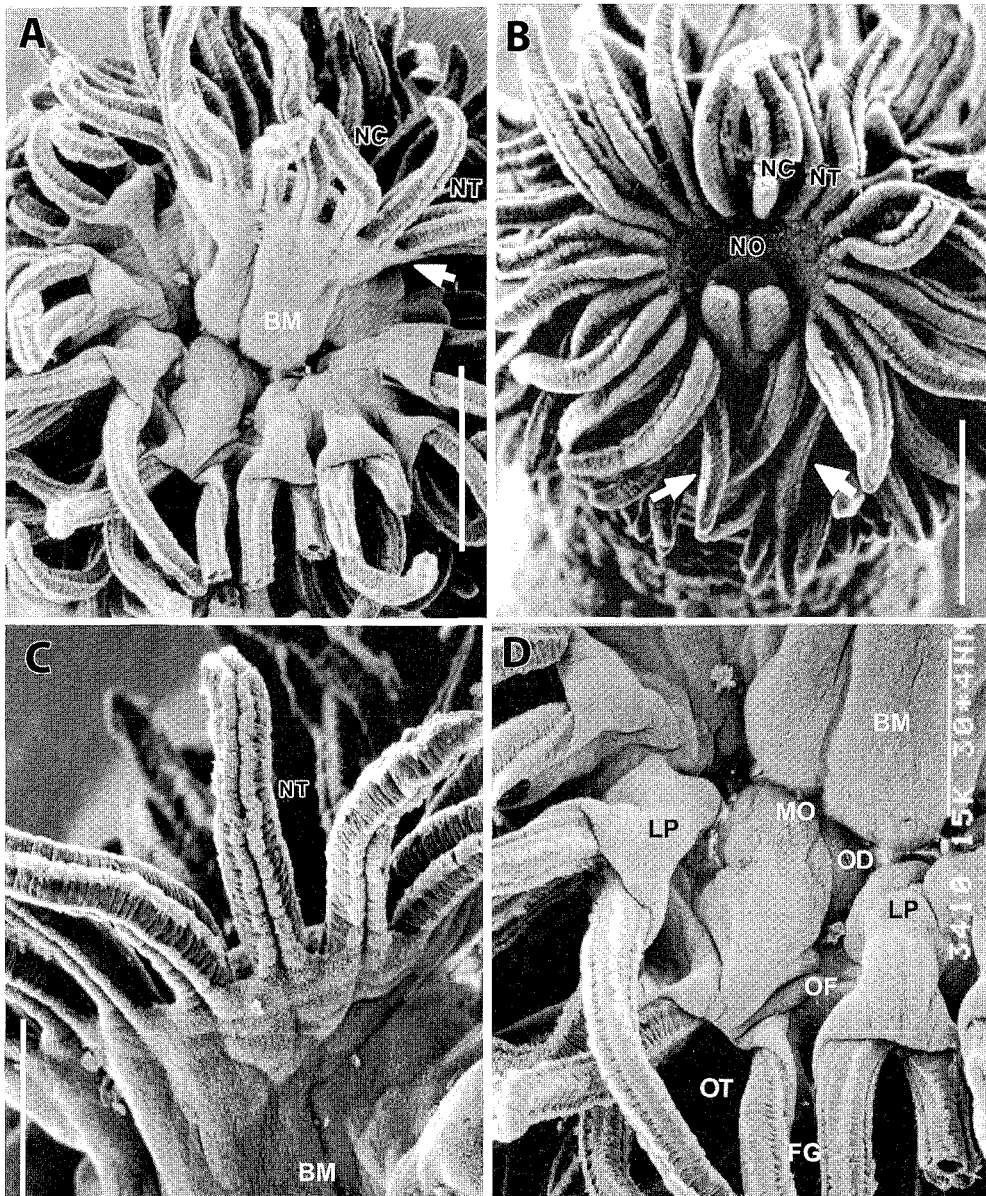


Fig. 2. Head of *Th. nigra*, scanning electron microscopy (SEM).

A - frontal view to oral disk and mouth;

B - dorsal view to nuchal organ and nuchal crown of tentacles;

C - nuchal tentacles;

D - oral tentacles and beginnings of oral festoons.

Bar: A - 0.6 mm, B - 0.5 mm, C - 0.25 mm, D - 0.3 mm.

Abbreviations: BM - ventral basement of nuchal crown, FG - festoon groove, LP - ciliar lips, MO - mouth opening, NC - nuchal (dorsal) crown of tentacles, NO - nuchal organ, NT - nuchal tentacle, OD - oral disk, OF - oral furrow, OT - oral tentacle. Arrow indicates the area where new dorsal tentacles appear.

The circular fold around the oral disk also bears numerous tentacles forming the oral crown (Figs. 1-2). The ciliar oral disk expands to posterior by radial growths bordered by paired rows of oral tentacles so as to form a circular series of U-shaped festoons (Fig. 1). New pairs of oral tentacles of grown animal appear in terminal (posteriormost) points of festoons. The elongating parallel festoons extend down (posterior) the oral disk and run alongside the spherical head (Fig. 1). A separate festoon of adult *Th. nigra* is composed of from 5 to 10-12 pairs of tentacles. Head of mature specimens usually bears 5-6 pairs of festoons thus 20-24 longitudinal rows (10-12 pairs) of oral tentacles may be counted for. A number of festoons increases with age as well as number of tentacles. Juvenile specimens possess only 3 pairs of festoons. New festoons appear pairwise (left and right ones) at the base of the dorsal arc, so a grown specimen have 4, 5 and 6 pairs of festoons respectively.

The tentacles are heart-shaped in cross section with a wide ciliated oral surface incised in midline by a longitudinal groove, and with a convex aboral surface (Figs. 3, 4 A). The nuchal tentacles face the oral disk directly by the oral surface while the oral tentacles arranged in festoons are twisted at the base, so to face a ciliary groove of the corresponding festoon (Fig. 2 D). Ciliary grooves of the nuchal tentacles run to furrows on the ventral side of the nuchal crown basement situated above the mouth. Ciliary grooves of the oral tentacles run to a groove of the corresponding festoon. In each festoon, opposed tentacles of every pair are slightly shifted to each other, so their opposed grooves also become shifted and never meet each other where they enter the festoon groove. Probably, it prevents that streams of food particles provided by oral cilia of opposed tentacles collide with each other to create

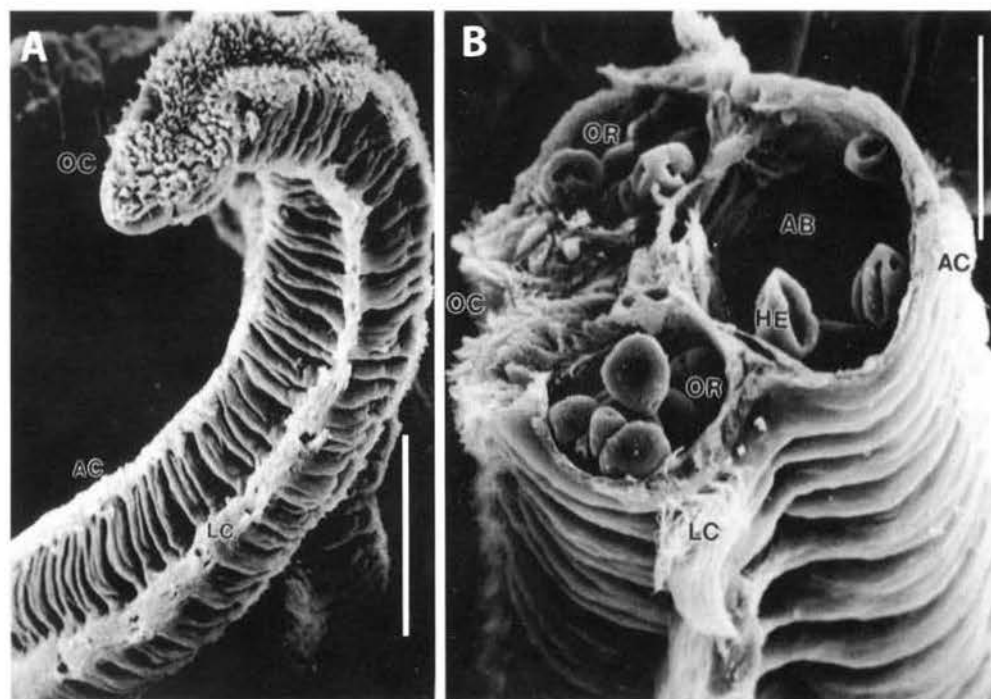


Fig. 3. Oral tentacles of *Th. nigra*, scanning electron microscopy (SEM). A - lateral view; B - cross section. Bar: A - 0.1 mm, B - 0.05 mm.

Abbreviations: AB - aboral canal, AC - aboral cilia, HE - hemerythrocytes, LC - lateral cilia, OC - oral cilia, OR - oral canal.

turbidity in the festoon groove. Festoon grooves run to the corresponding radial oral furrows which cross the ciliated oral disk and then enter the mouth opening (Fig. 1). A number of oral furrows is equal to the number of festoons and increases with age of the animal. Prominent swellings between these furrows are ciliary lips of the oral disk.

In a cross-section, the festoon groove is composed of a central groove bordered by prominent longitudinal lateral ridges (LR) with very long cilia (Fig. 4 B). Every longitudinal row of tentacles is accompanied by longitudinal ciliary ridge (AR) running alongside the aboral, or outer, side of festoon basement (Fig. 4 B). The central groove of festoon harbors a median ciliary ridge (MR) and two narrow longitudinal flutes situated between median and lateral ridges (Fig. 4 B).

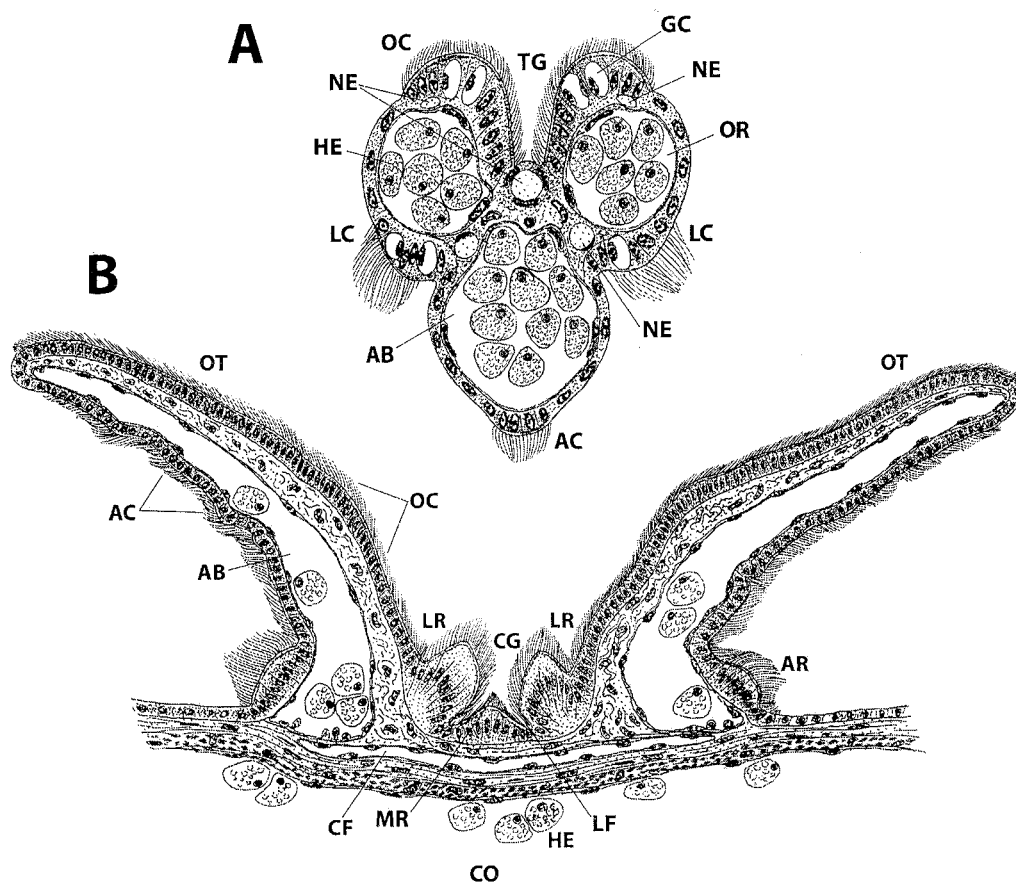


Fig. 4. Oral tentacles of *Th. nigra*, histological schemes. A - cross section of tentacle; B - cross section of festoon (sagittal section of tentacles).

Abbreviations: AB - aboral canal, AC - aboral cilia, AR - aboral ciliary ridge, CF - coelomic canal of festoon, CG - central groove of festoon, CO - coelom, GC - gland cell, HE - hemerythrocytes, LC - lateral cilia, LF - longitudinal flute of central groove, LR - lateral ciliary ridge of festoon, MR - median ciliary ridge of festoon, NE - tentacular nerve, OC - oral cilia, OR - oral canal, OT - oral tentacle, TG - tentacular groove.

In addition to the wide ciliary area on the oral surface, every tentacle bears two lateral and one aboral rows of very long cilia running alongside lateral and aboral surface correspondently (Fig. 3, 4 A). Ciliary beating is well seen in living specimens embedded into the water with suspension of small coelomocytes. Oral cilia of tentacles beat downward to bring food particles to the festoon groove. Instead, aboral cilia beat forward to the top of tentacle, while lateral cilia beat upward to the oral surface of tentacle (Fig. 5).

The oral surface of tentacles is constructed of a multiciliated, pseudostratified, columnal epithelium (Fig. 4 A). The aboral and lateral surface is composed of unciliated and ciliated, cuboidal epithelium (Fig. 4 A). Large mucous gland cells are present between the epithelial cells in the oral groove and near the junction of oral and lateral surfaces. These cells are heavily packed with large mucous droplets. Oral cilia are relatively short ($4.5\ \mu\text{m}$), while aboral and lateral cilia are much longer, 13-15 and 20-25 mm respectively (Figs. 3 B, 4 A).

Near each lobe of the triangular heart-shaped tentacle are three tentacular canals embedded within the extracellular matrix (Figs. 3 B, 4 A). These longitudinal canals pass through each tentacle, two

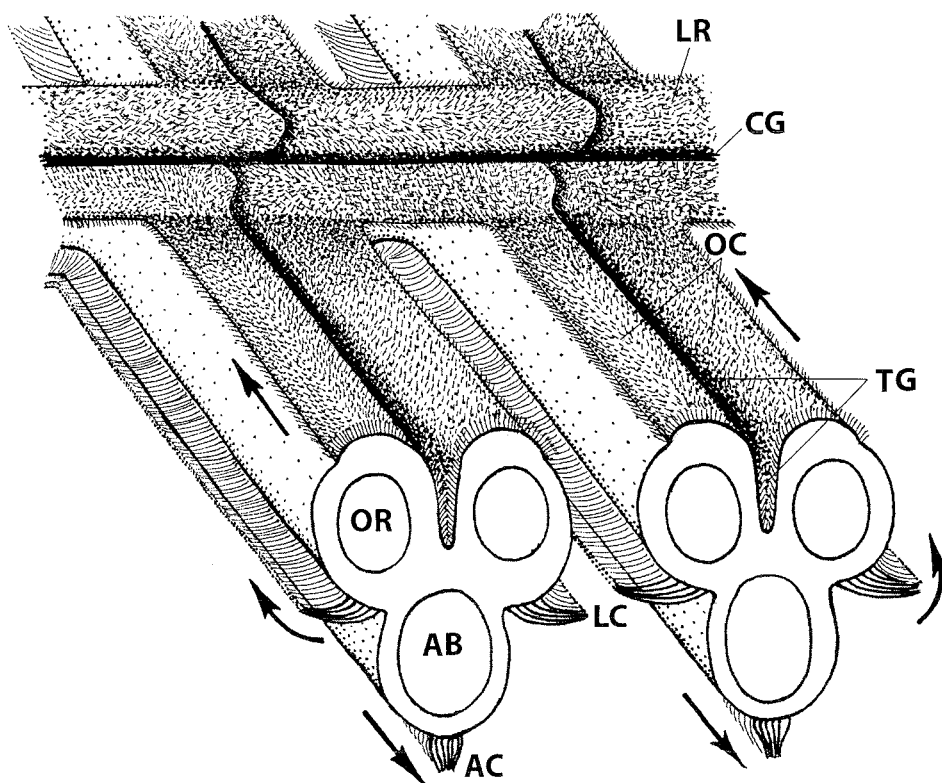


Fig. 5. Fragment of oral festoon of *Th. nigra*.

Abbreviations: AB - aboral canal, AC - aboral cilia, CG - central groove of festoon, LC - lateral cilia, LR - lateral ciliar ridge of festoon, OC - oral cilia, OR - oral canal, TG - tentacular groove. Arrows indicate direction of ciliary beating.

smaller oral canals (30 μm in diameter) and one larger aboral canal (40-50 μm in diameter) (Fig. 3 B). Each canal is fully lined by squamous peritoneum composed primarily of non-ciliated and multiciliated cells. The oral canals connect with the aboral canal at the tip of the tentacle to form a single terminal chamber.

Five tentacular nerves are embedded into a thick extracellular matrix beneath the epithelium. The main nerve cord is situated between two oral canals just in front of tentacular groove (Fig. 4 A). Two thin nerves lie at anterior margin of the oral canals, between basal infoldings of oral epithelial cells. Two other nerves are laterally to the aboral canal, between lateral epithelial cells (Fig. 4 A). Smooth muscles are also present in the extracellular matrix of the tentacles. Outer circular muscle cells are situated just beneath the tentacular epithelium being arranged in circular bundles around the tentacular canals. Inner longitudinal muscle cells lie between circular fibers and a peritoneal lining of the canals. A few strong bundles of longitudinal muscles are situated in the middle of tentacle, between canals and near the main tentacular nerve.

Tentacular canals are part of a complex system of the tentacular coelom and filled with numerous coelomocytes (Figs. 3 B, 4). The canals are lined by peritoneum composed of podocytes and multiciliary cells. Three types of freely floating coelomic cells - hemerythrocytes, amoebocytes and granulocytes are found within the lumen of tentacular canals. The flow of coelomic cells in the tentacle of living specimens may be easily seen with a microscope. They are carried in the fluid anterior, to the tip of the tentacle, in each of two oral canals and these passages are confluent with each other and with the aboral canal at the tip of the tentacle. The cells move posterior, to the base of the tentacle, through the single aboral canal. At the base of each nuchal tentacle the canals open into a system of interconnecting passages beneath the dorsal crown of tentacles. At the base of each oral tentacle three tentacular canals open into a longitudinal, or radial, coelomic canal running just beneath and alongside the corresponding festoon. These canals are connected with a spacious circumesophageal system of interconnecting passages.

Discussion

Sipunculans demonstrates three functional categories based on the body regions used for environmental gas exchange (Ruppert and Rice, 1995). "Integumentary breathers" are active burrowers, feeding on detritus in sand engulfed as they burrow and rarely emerging onto the surface. They are characterized by coelomic invaginations into the body wall, usually possess small tentacles and simple (non-branched) contractile vessel. "Introvert and tentacle breathers" typically extend the elongate introvert and moderately developed tentacles far into the surrounding water while the trunk remains lodged in calcareous rubble, rock fissures, shells or sand. "Tentacle breathers" with numerous well-developed tentacles and thick and dense body wall also inhabit sand, rock fissures, tunnels in coral rock, and their tentacles usually remain extended for long period over the substratum at the opening of the burrow or other refuge (see Ruppert and Rice, 1995). These species are also characterized by well developed contractile vessel elaborated into numerous diverticula.

Based on the structure of tentacular apparatus, habitus and behavior, *Th. nigra* is suggested to be a "tentacle breather". This large sand-burrowing species with numerous filiform tentacles and conspicuous branched villar projections on the long contractile vessel (see Adrianov and Maiorova, 2002) meets structural criteria for this category. *Th. nigra* usually extends its tentacular crown for long periods above the surface of fine muddy sand in which it burrows between roots of *Zostera marina* (see Adrianov *et al.*, 2001; Morozov and Adrianov, 2002). Manwell (1960) demonstrated that the hemerythrin of the tentacular system had lower oxygen affinity than that of the trunk coelom, suggesting that oxygen is transported from the sea-water through the tentacular system to the main coelom.

Ultrastructure of sipunculan tentacles has been reported only for *Themiste lageniformis* (see Pilger, 1982). Tentacles of *Th. nigra* differ from those of *Th. lageniformis* in having two lateral and

one aboral ciliar rows composed of very long cilia (about three times longer than oral cilia). These cilia create a water current around the crown and between adjacent tentacles to bring food particles from the outside to the oral surface of tentacles and to the oral disk. These particles are captured by the cilia of the oral surface of tentacles covered by adhesive mucous droplets produced by numerous glands of the oral epithelium.

Tentacular canals of *Th. nigra* are also part of the tentacular coelom composed of circumesophageal sinuses, radial canals, spacious ventral sinuses (reservoir) and contractile vessel (see Adrianov and Maiorova, 2002; Maiorova and Adrianov, 2003). In invertebrates, true blood vessels are invaginations of the basal lamina with the peritoneal cells into the coelomic cavity or spaces in the thick basal lamina underlying the peritoneum, and therefore they lack the endothelial lining that is characteristic of the invertebrates (Welsch and Storch, 1976). Likely in all other sipunculans, tentacular canals of *Th. nigra* are lined by peritoneum composed of podocytes and multicellular cells, and therefore the lumen constitutes a true coelomic space.

Th. nigra is suggested to be a filter feeder and their tentacular apparatus is beautifully adapted for the mucociliary suspension feeding. Sequential extrusion of individual mucous droplets by mucous cells of the tentacular oral epithelium is similar to that reported for *Themiste* (see Pilger, 1982) and known for polychaetes (see Storch and Welsch, 1972).

Tentacular apparatus of *Th. nigra* shows remarkable structural similarities with the tentacular apparatus of sabellid polychaetes, *Sabella*. Individual tentacles of *Th. nigra* corresponds structurally to the sabellid pinnulae situated along each tentacular filament of the branchial crown (Nicol, 1930). Each sabellid pinnula is characterized by the slightly incised oral, or frontal, surface with short oral cilia and also bears two lateral and one abfrontal (aboral) rows of long cilia. Each filament in *Sabella* bears two opposed rows of pinnulae and corresponds well with a sipunculan festoon (see Barrington, 1994, fig. 10-3). Pinnulae of adjacent filaments interlock to form an effective filtering system upon which food particles can be trapped. On the filament, sorting of food particles is provided by two continuous folds which are ciliary on both inner and outer sides. These folds border central ciliary groove of the filament to ensure that only suitable particles are directed into the mouth (Nicol, 1930; Barrington, 1994).

Feeding mechanism has never been described for *Thysanocardia* having a most complicated and extensive crown of tentacles. Now, based on morphology of the tentacular apparatus and vital observations on water currents created by cilia beat, the feeding and filtering mechanisms for *Th. nigra* can be suggested as follows (Fig. 5).

They obtain food by extending a crown of tentacles over the surface of the substratum and extract food particles from water currents, which are creating by coordinated cilia. Aboral cilia beat strongly toward the tip of each tentacle to draw water to the above of the "open festoon" (see Fig. 4 B). Lateral cilia, which are longest, beat upward to the oral surface of tentacle also to draw water from the outside into the festoon.

Because of the characteristic shape of the tentacular groove, a sorting process is suggested. Small particles from water currents fall into the narrow tentacular groove while large particles falling from above cannot enter the grooves and remain on the adhesive oral surface of tentacle. Both, small and large, particles trapped in mucus are driven by oral cilia to the base of tentacle where this groove incises the ciliar lateral ridge of the festoon and run into the festoon groove. Small particles easily enter into the corresponding longitudinal flutes between lateral and median ridges of the festoon groove along which these particles are propelled toward the oral disk. Large particles cannot enter through the narrow incision in the lateral ridge to enter the festoon groove. These particles running up against the lateral ridges are rejected by their long cilia finally being passed between the tentacles to the aboral sides of the festoon. Then, large particles are moved from between the neighboring festoons downward the head by beat of long cilia of continuous aboral ridges situated at the outer bases of the tentacular rows. Rejected large particles can also be moved away the head by beat of long aboral cilia of tentacles. Aboral cilia beat toward the top of tentacle but these large particles cannot again enter

into the festoon because of the presence of the subterminal gap between aboral and oral ciliar rows of the tentacle.

In the actively feeding specimen, adjacent tentacles in the festoon become close together and their lateral cilia can nearly interlock to form an additional filtering system where large particles cannot enter into festoon between these adjacent tentacles.

Because of the shape of festoon, additional sorting process is suggested in the festoon groove. Large particles falling from above cannot enter between close lateral ridges. Particles of medium size can pass between lateral ridges but cannot enter the narrow spaces (longitudinal flutes) between median and lateral ridges. Probably, these particles are carried toward the oral disk by cilia on the top of median ridge. Only small particles passing between lateral ridges can enter the narrow flutes between lateral and medium ridges and they are then propelled to the oral disk together with small particles coming from tentacular grooves. In *Sabella*, only small particles are ingested while medium-sized ones are used for building of the mud tube. *Th. nigra* does not construct any tubes and presently it is difficult to explain the reason to sort medium and small particles passing toward the oral disk.

Festoon grooves enter to the corresponding oral furrows which deeply incise the oral disk and pass suitable food particles to the mouth opening. Large particles, falling from above directly to the oral disk, are probably removed away from the disk through the action of rejection currents by the cilia of the prominent oral lips between the oral furrows.

Further investigations of the feeding behavior in *Thysanocardia* and other sipunculan worms are necessary to better understand functional morphology of the tentacular apparatus.

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